DOI: 10.7251/QOL2401005S
UDC: 665.347.8.035:621.646.4
Original scientific paper

Optimization of Oil Extraction Process from White Mustard Seeds Using Response Surface Methodology

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ABSTRACT: In this study, response surface methodology (*RSM*) was used for the determination of the effect of two process parameters (extraction temperature and extraction time) and their interaction on the yield of the total extract from white mustard seeds (*Sinapis alba l.*), as well as the creation of a mathematical model, 3D response surface, and the establishment of an optimal extraction region. Ethanol 96% as a green solvent was employed to separate biological compounds from white mustard (*Sinapis alba l.*) seeds by applying ultrasound-assisted extraction (*UAE*). *UAE* was performed at the temperature of 25, 30, and 35°C for a duration of 30, 60, and 90 min. *UAE* as a modern extraction technique showed great performance, while the ethanol solvent provided a high yield of compounds. The utilized *RSM* design adequately fit the experimental data with a high coefficient of determination (R^2=0.9365) and low mean absolute error (*MAE*=1.12242). Estimated effects for the yield of the total extract showed that the extraction time, the temperature, and the interaction between time and temperature influenced positively, while the time-squared and the temperature-squared influenced negativity on the yield of the total extract. Analysis of variance showed that 2 effects have *P*-values less than 0.05. The optimal yield of the total extracted oil was 15.19% at the temperature of 35°C for a duration of 70.3 min.

Keywords: white mustard seeds, extraction, *RSM* optimization, influence of process parameters, 3D response surface.

INTRODUCTION

Plants are sources of important bioactive compounds with diverse structures. Natural products strongly impact human culture and have been used as cosmetics, pigments, and pharmaceuticals (Alamgir, 2018). The extraction processes of natural products mainly have negative effects on the environment. The challenges launched by the competitiveness of the globalized market and environmental protection strongly require technological innovations. In this context, the development and use of green technologies for the separation of bioactive compounds from plant materials have essential meaning (Soquetta et al, 2018).

Different solid-liquid extraction techniques are used for the separation of biologically active compounds from white mustard (Barthet & Daun, 2002; Boscariol Rasera et al, 2019). The traditional solid-liquid extraction methods such as maceration (Swarcewicz et al, 2013) and Soxhlet extraction (Stamenković et al, 2018), have been associated with high consumption of organic solvents that limit the application of the extracts due to solvent toxicity. Additionally, these methods include high energy and time consumption (Picot-Allain et al, 2021). Ultrasound-assisted extraction (*UAE*) represents a sustainable alternative. *UAE* has certain advantages in terms of equipment cost, energy, and time consumption, application of green solvents, isolation of bioactive compounds under atmospheric pressure, etc (Wen et al, 2018). The application of ultrasound is relatively simple, flexible, and requires less investment compared to other extraction techniques. Therefore, this technique is characterized as green with low negative effects and impacts on the environment. *UAE* allows the use of all solvents, such as water or organic solvents (Chemat et al, 2017). However, according to the trends of green chemistry as solvents are used water, or a mixture of ethanol-water for the extraction of polar and oils for the extraction of non-polar compounds (Chemat et al, 2019). Ethanol (*EtOH*) is one of the most suitable solvents for green extraction. *EtOH* has a low vapor pressure, which leads to uncomplicated evaporation, and consequently lower inhaled quantities. *EtOH* is also cheaper than other organic solvents which promotes its use for the extraction of large amounts of natural products (Dogan et al, 2020).

Optimization, analysis, and control of extraction processes of bioactive compounds from plant materials are unavoidable phases for reducing energy exhaustion and reducing the influence on the environment (Iglesias-Carres et al, 2019; Rezazi et al, 2017). In addition, the use of natural extracts is becoming more popular than the use of chemically synthesized drugs. Conventional optimization involves the one-factor-at-time testing effect of operating parameters, while the other parameters are kept constant (Abdel-Rahman et al, 2020). However, statistically designed experiments in which several factors are varying simultaneously are more efficient when studying two or more factors (Li et al, 2016). In the last decade, response surface methodology (*RSM*) has been often used for modeling and mostly for optimization of various extraction processes and food manufacturing processes. This methodology as a statistical technique is useful for the determination of the influence of independent process parameters on dependent parameters. However, it is not able to use outside of the studied regions (Kasapoğlu et al, 2023; Krongrawa et al, 2022; Milić et al, 2013).

In this study, in order to optimize the ultrasound-assisted extraction of the yield of total separated biological compounds from white mustard seed by using ethanol 96%, as well as to determine and statistically verify the influence of two extraction process parameters on the investigated dependent variable, response surface methodology was implemented.

MATERIALS AND METHODS

Materials

The white mustard (*Sinapis alba l.*) seeds used in this study as a raw material for isolating natural compounds were purchased from Natural Pharmacy & Healthy Food (Skopje, N. Macedonia). The dry raw material was treated with a manual mince machine through holes with a diameter of 0.5 mm (particle size<0.5 mm), to homogenize the raw material and increase the contact surface. The material was stored in a plastic closed container at the ambient temperature of 20°C.

Ultrasound-Assisted Extraction Process

Ultrasound-assisted extraction (*UAE*) was performed in an ultrasonic bath (Ei Niš OOUR-RC). Ethanol 96% as a green solvent was used for the separation of biological compounds from white mustard (*Sinapis alba l.*) seeds. *UAE* was performed at a constant ultrasound frequency of 40 kHz and a constant module of sample-to-solvent ratio (raw material [g]:solvent [mL]=1:30). The white mustard seeds 3.00 g and 90 ml of ethanol 96% were placed into an Erlenmeyer flask with a volume of 300 mL. The flask was placed in the center of the ultrasonic bath. After the extraction process, the mixture was immediately filtered through a laboratory filter paper (pore size of 25 μm) using a vacuum filtration technique. The solvent was evaporated by using a rotary vacuum evaporator (Büchi R-200) at the temperature of 40°C until the entire solvent was removed from the extract. The obtained extract was dried in a laboratory furnace (Instrumentaria ST-06) at the temperature of 60°C and ambient air to constant mass. The dry residue represented the total extract from white mustard (*Sinapis alba l.*) seeds. *UAE* was employed because it is an effective technique for the extraction of compounds from plant materials. The acoustic cavitation enables better penetration of the solvent into the sample, increasing the extraction yield of target compounds. The efficiency of *UAE* depends on operation parameters, such as solvent concentration, solvent-to-solid ratio, extraction temperature, extraction time, and ultrasound frequency, as well as their interactions. Hence optimization is a very important phase in increasing product yield (Xu et al, 2016). Therefore in this study, the effects of extraction temperature and extraction time were investigated. *UAE* was carried out at the temperature of 25, 30, and 35°C for a duration of 30, 60, and 90 min.

The yield of the total extract was mathematically calculated by the equation:

$$Y_{total} = \frac{m_e}{m_s} 100 \quad (1)$$

where Y_{total} is the yield of the total extract [%], m_e is the mass of obtained extract [g], and m_s is the mass of raw material [g].

A non-linear equation was employed to develop the ultrasound-assisted extraction process kinetics model. The kinetics was mathematically defined by:

$$Y_{total} = \frac{at}{1 + bt} \quad (2)$$

where Y_{total} is the predicted yield of total extract, and a and b are regression coefficients. Regression coefficients were determined by using MATLAB-Curve Fitting.

RSM Design and Statistical Analysis

The response surface methodology (*RSM*) is a set of mathematical and statistical techniques useful for developing, improving, and optimizing processes. *RSM* is based on fitting experimental values using polynomial equations. This method is applied to investigate the influence of process parameters on one or more responses. *RSM* was employed to determine the effects of extraction time t and extraction temperature T and their interaction on the yield of the total extract Y_{total} from white mustard (*Sinapis alba l.*) seeds using ethanol 96%. The *RSM* model was developed using Statgraphics Centurion XV. The 3^2 full factorial design was employed for the optimization of the process. The experimental data for the yield of total extract was fitted using a second-order polynomial equation and the regression coefficients were calculated. The model was mathematically defined using the following equation:

$$Y_{total} = k_0 + k_1 t + k_2 T + k_3 t^2 + k_4 tT + k_5 T^2 \quad (3)$$

where Y_{total} is the response or the predicted yield of the total extract [%], t is extraction time [min] and T is extraction temperature [°C]. The adequacy of the applied method was determined by the coefficient of determination (R^2) and the mean absolute error (*MAE*) (Kuvendziev et al, 2014):

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (Y_i - Y_{ai})^2}{\sum_{i=1}^{n} (Y_{ai} - Y_m)^2} \quad (4)$$

$$MAE = \frac{1}{n} \sum_{i=1}^{n} |Y_i - Y_{ai}| \quad (5)$$

where n is the number of points, Y_i is the predicted value, Y_{ai} is the experimental value, and Y_m is the average of the experimental values.

RESULTS AND DISCUSSION

Effect of Process Parameters

From several operating parameters to influence the yield of total extract, the temperature T and the extraction time t were determined as independent operating parameters for optimization of the ultrasound-assisted extraction of biological compounds from white mustard seeds using a green polar solvent.

In order to study the effect of the temperature (25-35°C) and extraction time (0-90 min) on the yield of total extract, other parameters were kept fixed. The modeled kinetics of the studied process is given in Figure 1. The regression coefficients of the kinetic models are given in Table 1.

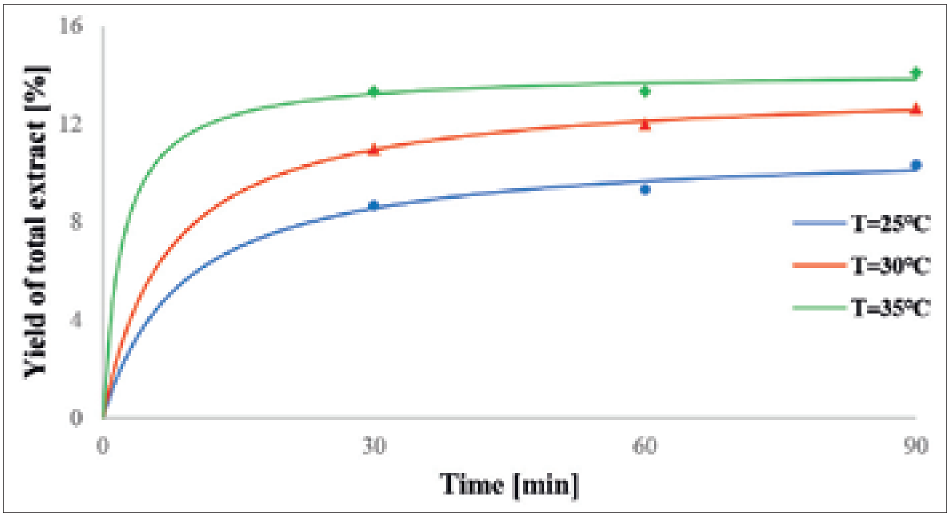

Figure 1. Kinetic of the yield of total extract from white mustard seeds

Table 1. Regression coefficients of developed kinetic models

T [°C]	*a*	*b*	R^2
25	1.2460	0.1122	0.9974
30	1.8950	0.1396	0.9998
35	6.7280	0.4750	0.9985

The experimental results suggested that the extraction time and extraction temperature have a greatly positive effect on the yield of biological compounds from white mustard (*Sinapis alba l.*) seeds. *RSM* was employed for creating the optimization model for the investigated system.

RSM Optimization

RSM was used for the determination of the influence of working parameters and their interaction on the yield of the total extract, as well as the creation of a mathematical model, 3D response surface, and the establishment of an optimum extraction region. The Pareto chart (significance level, α=0.05) of the importance and influence of the independent operating parameters (*t* and *T*) on the Y_{total} is presented in Figure 2. Each of the estimated effects is given in Table 2.

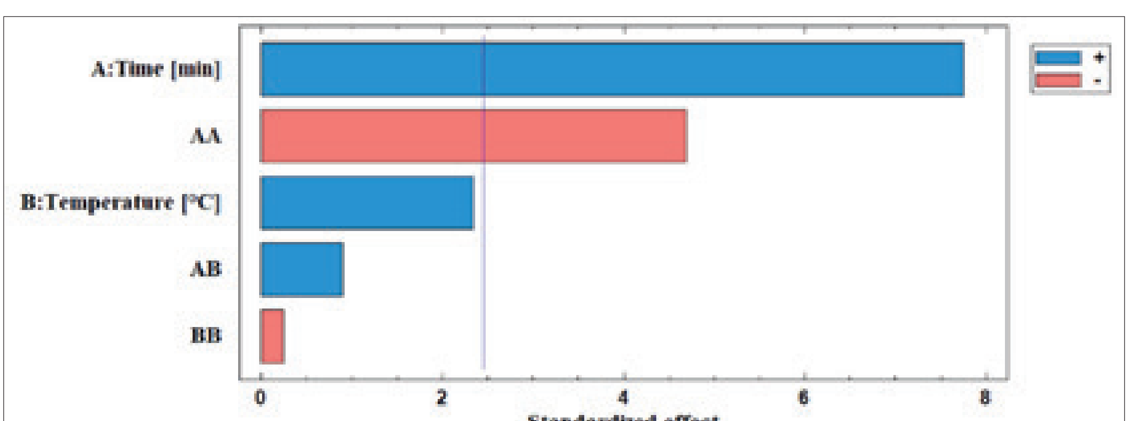

Figure 2. Pareto chart of standardized effect of studied experimental factors for ultrasound-assisted extraction, $Y_{total}=f(t,T)$

Table 2. Estimated influence of operating parameters on Y_{total} for *UAE*

Variables	Estimated effect
Average	12.1001
Time	11.2966
Temperature	3.10675
Time-squared	-11.46
Temperature-squared	-0.56025
Time x Temperature	1.59525

The Pareto chart showed that the extraction time (A), the temperature (B), and the interaction between extraction time and temperature (AB) influence positively, while the time-squared (AA) and the temperature-squared (BB) have a negative influence on the yield of the total extract. Analysis of variance (AVONA) which tests the statistical significance of each effect by comparing the mean square against an estimate of the experimental error, showed that 2 effects (A and AA) have *P*-values less than 0.05. The impacts of the extraction time and the temperature on the response of interest are given graphically in Figure 3.

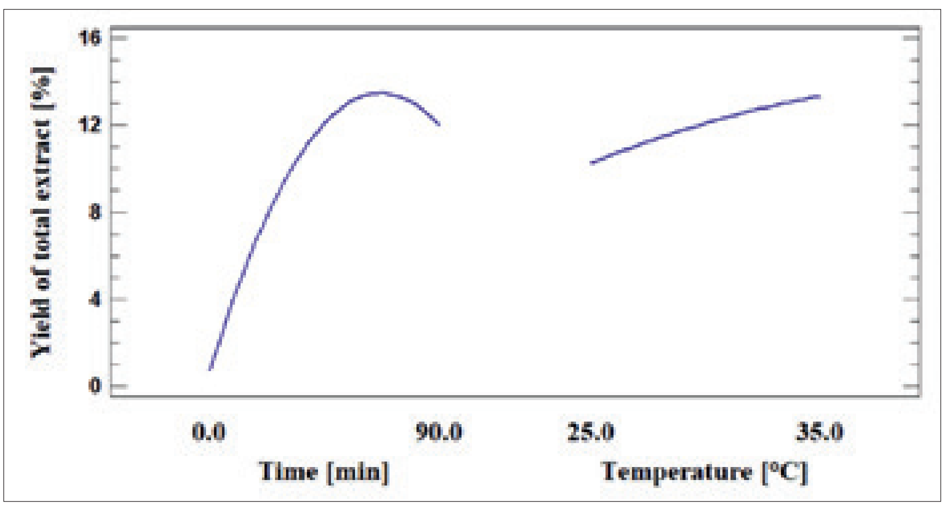

Figure 3. Main effects plot for the yield of total extract

The second-order empirical equation was utilized for the mathematical definition of the dependence of the yield of the total extract on the extraction time and extraction temperature according to Equation 3. The regression coefficients are given in Table 3.

Table 3. Regression coefficients of the *RSM* model

Coefficient	Value
k_0	-13.8972
k_1	0.273834
k_2	0.82345
k_3	-0.00282963
k_4	0.003545
k_5	-0.011205

The standard error of the estimate showed the standard deviation of the residuals was 1.88074, and the mean absolute error (*MAE*) was 1.12242. The *RSM* provided an adequate fitting of experimental data with a high coefficient of determination (R^2) of 0.9365. In an aim to graphically demonstrate the influence of investigated process parameters on the yield of total extract, the 3D response surface was created (Figure 4).

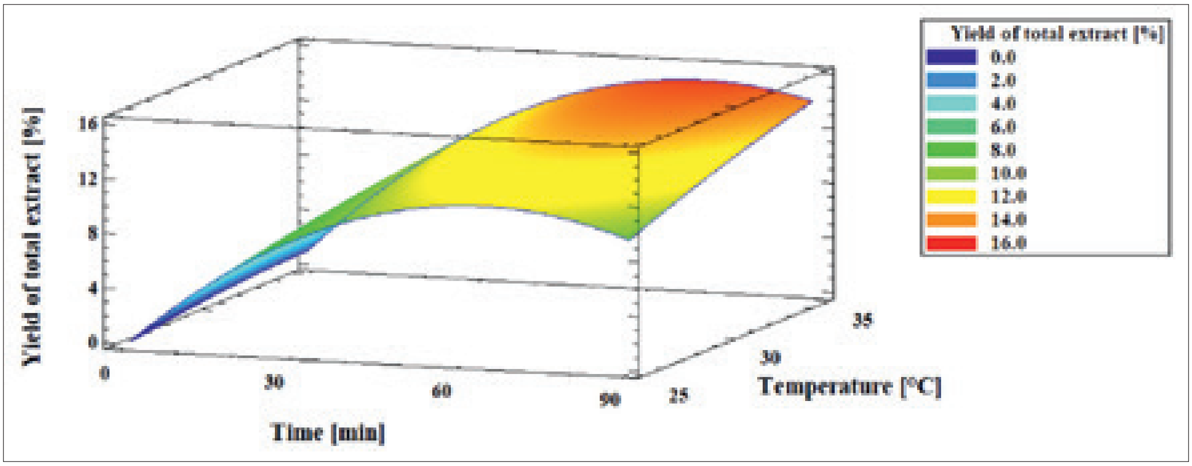

Figure 4. Estimated 3D response surface for the ultrasound-assisted extraction of the yield of total extract using ethanol 96%; $Y_{total}=f(t, T)$

The 3D response surface showed that in the observed ultrasound-assisted extraction system, the investigated operating parameters have significant effects on the total separated compounds from white mustard seeds, extracted by using green solvent. The developed 3D response surface showed that extraction time has a high effect on the yield of total extract in the first 30 minutes i.e. washing step. The generated response graph demonstrated that in the diffusion step, increasing the operating temperature in the range of 25–35°C results in a linear increase in the yield of the total extract. This phenomenon is not detected by the extraction time increasing.

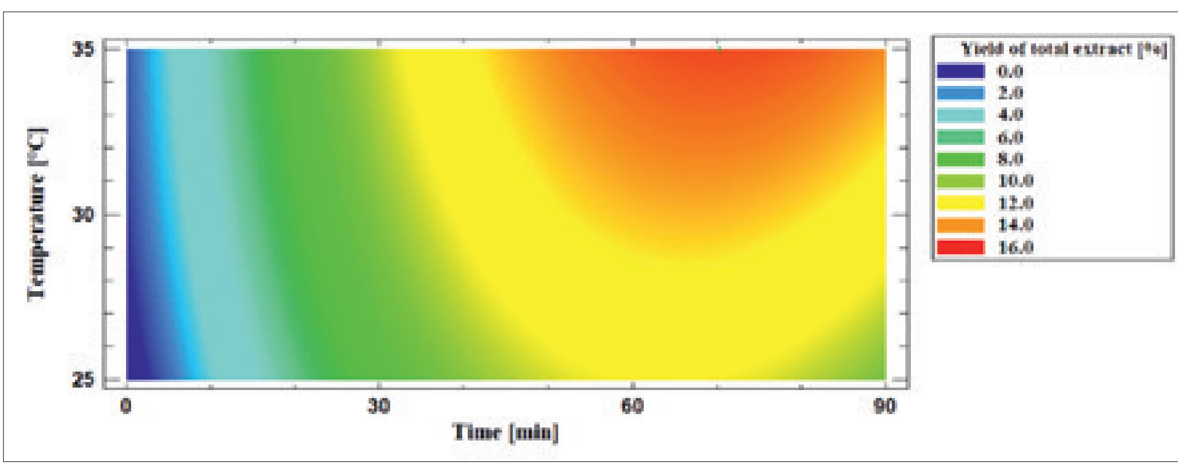

Figure 5. Contours of an estimated response surface for the *UAE*; $Y_{total}=f(t, T)$

The created contours of the estimated response surface (Figure 5) show that the optimum value of yield of the total extract is 15.19% at the temperature of 35°C and extraction time of 70.3 min.

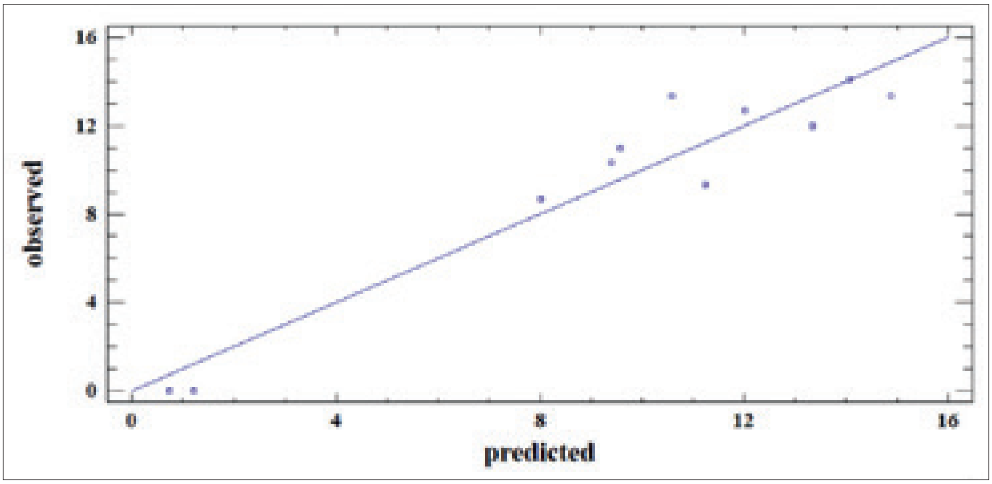

Figure 6. The plot of *RSM* predicted versus observed values for *UAE*; $Y_{total}=f(t, T)$

The *RSM* was applied for the prediction of the actual yield obtained from white mustard seed. The comparison is given in Figure 6.

CONCLUSION

The process of ultrasound-assisted extraction of biological compounds from white mustard seed using green solvent (ethanol 96%) was investigated and optimized by observing the effects of extraction time and temperature on the yield of the total extract. *UAE* as a modern extraction technique showed great performance, while the ethanol solvent provided a high yield of compounds. From a technological point of view, lower temperatures and shorter time of extraction were investigated. The *RSM* provided an adequate fitting of the experimental data with a high coefficient of determination (R^2=0.9365) and low mean absolute error (*MAE*= 1.12242). The model showed that the extraction time is more important than the extraction temperature. However, these parameters had a complex impact. The optimal yield of the total extracted oil was 15.19% at the temperature of 35°C for a duration of 70.3 min.

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Recived: June 14, 2023
Accepted: September 16, 2023